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To: C. Ellis **Date:** September 21, 1990

From: R. McCuen

Subject: **Abstract of Planning Meeting Presentation - September 17, 1990**

Dr. McCuen presented the Lowered Biological (LBA) Program - the first such presentation since March of 1989. The objective and strategies for this effort remained unchanged - to reduce the biological activity of CSC by 90% in at least one ***in vitro*** bioassay.

Dr. McCuen covered the bioassay development major strategy by focusing on the EGF binding, JB6 cell transformation and glutathione depletion assays. The EGF system was shown to respond to catechol. Removal of catechol from CSC was accomplished with the enzyme catechol oxidase. While enzyme-treated CSC did enhance EGF binding (a positive finding) so did heat inactivated enzyme-treated CSC and enzyme alone. The latter two results made interpretation of the first result very difficult. With the JB6 cell transformation system which was established at PM using TPA, a positive response has not been obtained with CSC. This was despite examination of several variables in the assay - serum supplier/concentration, source of water, length of treatment time, etc. The GDA which does respond to CSC has not been used to any great extent to evaluate models. This is because we have been unable to show the biological relevance of a CSC glutathione depleted cell versus a non-depleted cell. In other words, mutagenicity in GSH depleted ***Salmonella*** or V79 cells was no greater than in cells which had not been depleted of GSH.

In the model development and evaluation major strategy, Dr. McCuen discussed a variety of attempts to manipulate burley tobacco solubles to lower S/M bioactivity. Of the experiments conducted - calcium acetate, sugars (glucose/fructose/sucrose), amino acids (lysine/proline/arginine), hydrogen peroxide and chelation using silver nitrate - only the chelation efforts were successful in reducing biological activity by about 55% versus a suitable control. Two ongoing studies are part of this strategy. The first was a hollow fiber membrane ultrafiltration of burley solubles to remove materials based on molecular weight. The experiments have been done and the cigarettes made; however, the biological activity has yet to be determined. The second study used electrodialysis to remove ions of interest from the burley solubles. Initial attempts have focused on nitrate removal (which was successful) and then an add-back of different nitrogen sources to ascertain how they affect biological activity.

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PM has one bioassay (S/M) that measures initiating type compounds in CSC. No *in vitro* assay specific for promoters in CSC has been found. In an attempt to lower the bioactivity of burley tobacco solubles as measured in the S/M assay, only two methods have been successful - strong cation exchange column chromatography (70% reduction) and chelation with silver nitrate (55% reduction).

During the course of his presentation, several questions were fielded from the attendees, some of which are summarized below.

Walter Hempfling suggested that catechol oxidase could be immobilized on a support and used in a column mode with CSC. This should reduce the EGF binding enhancers apparently present in the enzyme preparation from getting into the CSC. This idea will be tested.

Richard Carchman suggested that the residual glutathione levels (~30% of the starting concentration) in the microbial or mammalian cells following depletion may be enough to protect the cell against a mutagenic insult. This is reasonable, but the assay is being conducted that the maximum limit of depletion and mutation so as to avoid toxicity. A dead cell does not respond to either parameter measured. Richard also wanted to know if in the EGF binding experiments where enhancement was observed was the enhancement due to an increased number of EGF receptor sites or an increased affinity of the receptor for the ligand. Maybe these analyses would distinguish among the kinds of increased EGF binding observed for catechol-depleted CSC, heat-inactivated catechol-depleted CSC and when the enzyme was tested alone.

Andy Kallianos was interested in the successful CSBW remake and whether the burley solubles obtained via centrifugation of BuCEL were similar (chemical/biological activity). This is currently being examined to determine the effect of storage time on burley solubles.

Ken Houghton queried as to the chemical differences between impaction trapped CSC and cold trapped CSC as the different trapping methods may be affecting the lack of response in the JB6 cell transformation system. John Lephardt indicated there were some differences in the nitrogen-containing compounds such as the nitroarylamines.

/mps

Bob McCrea

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